Application No. 10/730,549 Docket No.: CWRU-P01-046
Amendment dated March 13, 2007

Reply to Office Action of November 13, 2006

AMENDMENTS TO THE CLAIMS

1. (Currently Amended) A method for treating an ischemic tissue in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of enriched human endothelial generating cells and enriched human mesenchymal stem cells, wherein the endothelial generating cells are enriched from bone marrow mononuclear cells at least two-fold prior to administration to the subject.

- 2. **(Previously presented)** The method of claim 1, wherein the human endothelial generating cells are human hemangioblast cells.
- (Currently Amended) The method of claim 1, wherein the endothelial progenitor
 generating cells are generated in culture from hematopoietic stem cells, hemangioblasts or
 embryonic stem cells.
- 4. (Original) The method of claim 1, wherein treatment of the ischemic tissue induces
 - (a) formation of blood vessels supplying blood to the ischemic tissue;
 - (b) blood flow to the ischemic tissue;
 - (c) oxygen supply to the ischemic tissue; or
 - (d) a combination thereof.
- 5. (Withdrawn-Currently Amended) A method for treating an ischemic tissue in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of enriched human endothelial generating cells and enriched human mesenchymal stem cells The method of claim 1, wherein the endothelial generating cells are isolated from umbilical cord blood or from peripheral blood.

6-7. (Canceled)

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- 8. (Currently Amended) The method of claim 1, wherein the endothelial generating cells are culture expanded, under endothelial cell-promoting culture conditions that promote the formation of endothelial cells, prior to administration to the subject.
- 9. (Withdrawn) The method of claim 1, wherein the endothelial generating cells are autologous to the subject.
- 10. (Previously presented) The method of claim 1, wherein the endothelial generating cells are allogeneic to the subject.
- 11. (Original) The method of claim 1, wherein the endothelial generating cells are HLA compatible with the subject.
- 12. (Currently amended) The method of claim 1, wherein the endothelial generating cells are CD31⁺, CD146⁺, CD133⁺, CD34⁺, VE-cadherin⁺ or a combination thereof.
- 13. (Original) The method of claim 1, wherein the endothelial generating cells are CD133⁺.
- 14. (Original) The method of claim 1, wherein the endothelial generating cells are CD34⁺.
- 15. (Canceled)
- 16. **(Previously presented)** The method of claim 1, wherein the endothelial generating cells are endothelial progenitor cells, hemangioblasts or hematopoietic stem cells, or a combination thereof.
- 17. (Original) The method of claim 1, wherein the human mesenchymal stem cells are isolated from bone marrow.

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18. (Withdrawn) The method of claim 1, wherein the human mesenchymal stem cells are isolated from umbilical cord blood.

19. (Previously presented) The method of claim 1, wherein the human mesenchymal stem

cells are culture-expanded prior to administering the human mesenchymal stem cells to the

subject.

20. (Currently Amended) The method of claim 19 1, wherein the human mesenchymal stem

cells are culture-expanded human mesenchymal stem cells to enrich for cells containing

surface antigens identified by monoclonal antibodies SH2, SH3 or SH4, prior to

administering the human mesenchymal stem cells to the subject.

21. (Previously presented) The method of claim 1, wherein the human mesenchymal stem

cells are autologous to the subject.

22. (Withdrawn) The method of claim 1, wherein the human mesenchymal stem cells are

allogeneic to the subject.

23. (Original) The method of claim 1, wherein the human mesenchymal stem cells are HLA

compatible with the subject.

24. (Previously presented) The method of claim 1, wherein the therapeutically effective

amount of enriched human endothelial generating cells and enriched human mesenchymal

stem cells is a safe amount.

(Original) The method of claim 1, wherein the therapeutically effective amount of enriched 25.

human endothelial generating cells comprises at least 1 x 10⁴ human endothelial generating

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cells.

- 26. (Previously presented) The method of claim 1, wherein the therapeutically effective amount of enriched human endothelial generating cells comprises between 1 x 10⁴ to 5 x 10⁸ human endothelial generating cells.
- 27. (Previously presented) The method of claim 2, wherein the therapeutically effective amount of the hemangioblast cells and the human mesenchymal stem cells is a minimum number of cells necessary for increased blood flow induction to the ischemic tissue.
- 28. (Previously presented) The method of claim 1, wherein the human endothelial generating cells and the human mesenchymal stem cells are administered in a ratio of from about 5:1 to about 1:5.
- 29. (**Original**) The method of claim 1, comprising administering to the subject a systemic infusion of the human endothelial generating cells.
- 30. (Previously presented) The method of claim 29, wherein the infusion is into bone marrow.
- 31. (Original) The method of claim 1, wherein administering to the subject comprises an intraarterial infusion of the human endothelial generating cells.
- 32. (**Original**) The method of claim 1, wherein administering to the subject comprises an intracardiac infusion of the human endothelial generating cells.
- 33. (Original) The method of claim 1, administering to the subject comprises an intracoronary infusion of the human endothelial generating cells.

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- 34. (Original) The method of claim 33, wherein said subject is in need of treatment for chronic myocardial ischemia.
- 35. (Original) The method of claim 1, wherein administering to the subject comprises using an intra-arterial catheter or a stent.
- 36. (Previously presented) The method of claim 1, wherein said subject is in need of treatment for ischemia selected from limb ischemia, ischemic cardiomyopathy, myocardial ischemia, cerebrovascular ischemia, renal ischemia, pulmonary ischemia and intestinal ischemia.
- 37. (Withdrawn) The method of claim 1, wherein the human endothelial generating cells are genetically modified.
- 38. (Withdrawn) The method of claim 37, wherein the human endothelial generating cells are genetically modified to express a recombinant polypeptide.
- 39. (Withdrawn) The method of claim 38, wherein the recombinant polypeptide is VEGF, BFGF, SDF, CXCR-4 or CXCR-5.
- 40. (**Original**) The method of claim 1, further comprising administering to the subject at least one recombinant polypeptide.
- 41. (**Original**) The method of the claim 40, wherein the recombinant polypeptide is VEGF, BFGF, SDF, CXCR-4 or CXCR-5.

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42. (**Original**) The method of claim 38 or 40, wherein the recombinant polypeptide promotes angiogenesis, vasculogenesis, or both.

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- 43. (Currently amended) The method of the claim 38 or 40, wherein the recombinant polypeptide is selected from among a growth factor, a cytokine, a chemokine or a receptor thereof.
- 44. (Canceled)
- 45. (Currently Amended) The method of claim [[44]] 48, wherein the endothelial precursor cells are CD133⁺ human endothelial precursor cells.
- 46. (Currently Amended) The method of claim 1 or [[44]], wherein the endothelial precursor cells are CD34⁺ human endothelial precursor cells.
- 47. (Currently Amended) The method of claim [[44]] 48, wherein the endothelial generating cells are culture-expanded under endothelial cell-promoting culture conditions prior to administration to the subject.
- 48. (Withdrawn-Currently Amended) A method for increasing blood flow to an ischemic myocardium in a subject in need hereof, comprising administering to the subject a therapeutically effective amount of enriched human endothelial precursor cells and enriched human mesenchymal stem cells The method of claim 44, wherein the endothelial precursor cells are isolated from umbilical cord blood.
- 49. (Currently Amended) The method of claim [[44]] 48, wherein the human mesenchymal stem cells are expanded in culture prior to administration to the subject.

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- 50. (Currently Amended) The method of claim [[44]] 48, wherein the human endothelial precursor cells and the human mesenchymal stem cells are administered to the subject by infusion into at least one coronary artery.
- 51. (Currently Amended) The method of claim [[44]] 48, wherein said ischemic myocardium comprises an area of viable myocardium.
- 52. (Currently Amended) The method of claim [[44]] <u>50</u>, wherein the coronary artery is an epicardial vessel that provides collateral blood flow to said ischemic myocardium in the distribution of a chronic totally occluded vessel.
- 53. (Currently Amended) The method of claim [[44]] 48, wherein the endothelial precursor cells and the mesenchymal stem cells are administered in a ratio from about 5:1 to about 1:5.
- 64. (Currently Amended) A method for improving blood flow to an ischemic myocardium having an area of viable myocardium in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of enriched CD133⁺/CD34⁺ cells isolated from umbilical cord blood, wherein the enriched CD133⁺/CD34⁺ cells are administered by infusion into a coronary artery that is an epicardial vessel that provides collateral flow to said ischemic but viable myocardium in the distribution of a chronic totally occluded vessel, and wherein administering of the CD133⁺/CD34⁺ cells results in improved blood flow to said ischemic myocardium, wherein the-CD133⁺/CD34⁺ cells are enriched from bone marrow mononuclear cells at least two-fold prior to administration to the subject.
- 55. (**Original**) The method of claim 54, further comprising administering to the subject enriched human mesenchymal stem cells.
- 56. (Original) The method of claim 54, wherein the human mesenchymal stem cells are

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isolated from said subject.

57. (Currently Amended) A method for inducing the formation of blood vessels in an ischemic tissue in a subject in need thereof, comprising administering to said subject a therapeutically effective amount of enriched human CD133⁺/CD34⁺ hemangioblast cells and enriched human mesenchymal stem cells, wherein the human CD133⁺/CD34⁺ hemangioblast cells are enriched from bone marrow mononuclear cells at least two-fold prior to administration to the subject.

58-61. (Canceled)

- 62. (Currently Amended) The method of claim 4 5, wherein the wherein the enriched endothelial generating cells (i) are enriched CD133+ hemangioblasts purified from umbilical cord blood; and (ii) are allogeneic to the subject.
- 63. (Currently Amended) The method of claim 62, wherein the enriched CD133+
 hemangioblasts and the enriched human mesenchymal stem cells are administering
 administered to a subject via intracoronary infusion, and wherein the subject is afflicted with
 myocardial ischemia-via intracoronary infusion.
- 64. (New) The method of claim 1, comprising administering to the subject from 10^4 to $5x10^8$ mesenchymal stem cells.
- 65. (New) The method of claim 1, wherein the endothelial generating cells have been enriched from isolated mononuclear cells.
- 66. (New) The method of claim 65, wherein the endothelial generating cells have been enriched from isolated mononuclear cells by immunoselection.

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67. (New) The method of claim 28, wherein the human endothelial generating cells and the human mesenchymal stem cells are administered in a ratio of from about 2:1 to about 1:2.

68. (New) The method of claim 67, wherein the human endothelial generating cells and the

human mesenchymal stem cells are administered in a ratio of about 1:1.

69. (New) The method of claim 10, wherein the human mesenchymal stem cells are autologous

to the subject.

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